



Llanos-Cuentas, A; Echevarria, J; Seas, C; Chang, E; Cruz, M; Alvarez, E; Rosales, E; Campos, P; Bryceson, A (2007) PARENTERAL AMINOSIDINE IS NOT EFFECTIVE FOR PERUVIAN MUCOCUTANEOUS LEISHMANIASIS. *The American journal of tropical medicine and hygiene*, 76 (6). pp. 1128-1131. ISSN 0002-9637

Downloaded from: <http://researchonline.lshtm.ac.uk/9845/>

DOI:

#### Usage Guidelines

Please refer to usage guidelines at <http://researchonline.lshtm.ac.uk/policies.html> or alternatively contact [researchonline@lshtm.ac.uk](mailto:researchonline@lshtm.ac.uk).

Available under license: Copyright the publishers

## PARENTERAL AMINOSIDINE IS NOT EFFECTIVE FOR PERUVIAN MUCOCUTANEOUS LEISHMANIASIS

ALEJANDRO LLANOS-CUENTAS, JUAN ECHEVARRIA, CARLOS SEAS,\* ELSA CHANG, MARIA CRUZ, EUGENIA ALVAREZ, ERNESTO ROSALES, PABLO CAMPOS, AND ANTHONY BRYCESON

*Instituto de Medicina Tropical Alexander Von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru; Departamento de Enfermedades Infecciosas, Tropicales y Dermatológicas, Hospital Nacional Cayetano Heredia, Lima, Peru; Departamento de Medicina, Facultad de Medicina Alberto Hurtado, Universidad Peruana Cayetano Heredia; Departamento de Medicina del Hospital Nacional Sur Este, Essalud Cuzco, Perú; Hospital Regional del Cuzco, MINSA, Peru; Facultad de Salud Pública y Administración, Universidad Peruana Cayetano Heredia, Lima, Peru; London School of Hygiene and Tropical Medicine, United Kingdom*

**Abstract.** Few therapeutic options are available for mucocutaneous leishmaniasis (MCL). We conducted a randomized open trial to evaluate the efficacy, safety, and tolerance of parenteral aminosidine sulphate (AS) 14 mg/kg/d for 21 days compared with intravenous meglumine antimonate (MA) 20 mg/kg/d for 28 days in patients with moderate MCL in Cuzco, Peru. Cure was defined as complete healing with re-epithelialization within 1 year of follow-up. The trial was stopped after 38 patients were enrolled (17 in the MA group and 21 in the AS group) because of marked differences in response. Study groups were comparable in baseline characteristics. Cure rates were 0/21 in the AS group compared with 8/17 (47%, 95% confidence interval: 23–71%) in the MA group ( $P < 0.001$ ). Side effects and laboratory abnormalities were mild in both groups. We conclude that parenteral AS given on its own is not effective for MCL in Peru.

### INTRODUCTION

Mucocutaneous leishmaniasis (MCL) caused by *Leishmania (Viannia) braziliensis* is a significant health problem in rural areas of Central and South America.<sup>1,2</sup> MCL is endemic in Peru along the eastern slopes of the Andes and throughout the Amazon jungle, where prevalence rates in certain areas are as high as 339 cases per 100,000 inhabitants.<sup>3</sup> MCL is a chronic illness that affects primarily the nasal and oropharyngeal mucosa, but if left untreated may extend to the larynx, causing significant morbidity and occasionally mortality. MCL also carries social, aesthetic, and economic problems.<sup>4</sup>

Few drugs are available to treat MCL. The first-line drugs are pentavalent antimonials, which have to be administered parenterally over a long time, are associated with toxicity, attain modest clinical cure rates especially in patients with extensive mucosal involvement, and are expensive.<sup>4–7</sup> Cure rates up to 70% are achieved in patients with nasal or oropharyngeal involvement, but only 10–63% in patients with laryngeal involvement.<sup>8–10</sup> Patients refractory to antimonials or with extensive mucosal involvement are treated with repeated cycles of pentavalent antimonials or with a second-line drug. Amphotericin B deoxycholate is still the preferred second-line drug.<sup>1,11</sup> Treatment with amphotericin B requires hospitalization and continuous monitoring for toxicity.<sup>12</sup> Other second-line drugs such as pentamidine, allopurinol, and the azoles are much less effective and share the problems of cost, toxicity, and need for hospitalization and monitoring.<sup>13–15</sup>

Aminosidine is an aminoglycoside antimicrobial agent chemically similar to neomycin, with *in vitro* activity against bacteria, intestinal tapeworms, enteric protozoan parasites, and several species of *Leishmania*.<sup>7,16,17</sup> Topical preparations of aminosidine alone or combined with systemic antimonials attained good clinical efficacy against agents of new and old world cutaneous leishmaniasis (CL).<sup>18–20</sup> Excellent clinical efficacy rates have been reported from India more recently in patients with visceral leishmaniasis with the parenteral for-

mulation, aminosidine sulphate.<sup>21,22</sup> Few data are available on the efficacy of aminosidine for treating MCL.<sup>23</sup> We therefore undertook this study to evaluate the efficacy, safety and tolerability of aminosidine sulphate in treating moderate MCL and to compare it with meglumine antimonate.

### MATERIALS AND METHODS

**Study design and criteria for enrollment.** The study followed a randomized and open label design and was conducted in the city of Cuzco, Peru, from October 1993 to May 1994. Ethical approval was obtained from the Special Program for Research and Training in Tropical Diseases of the World Health Organization and from Universidad Peruana Cayetano Heredia's Institutional Review Board. Patients gave written consent to participate.

Eligible patients were adults between 18 and 60 years of age with moderate MCL, defined as involvement of the nasal and pharyngeal mucosa with or without laryngeal affection but without respiratory distress and with proven presence of parasites by culture, histology, and/or polymerase chain reaction (PCR) on a biopsy specimen.<sup>24</sup> Patients who had received treatment in the previous 6 months with anti-leishmanial agents or who had failed to a course of treatment with amphotericin B were excluded, as well as patients with known or suspected allergy to aminoglycosides or antimonials, pregnant or nursing women, and patients not willing to return for follow-up evaluations. We also excluded patients with severe concurrent illnesses such as tuberculosis, renal, liver, or heart disease, or alcoholism. Clinical evaluation of patients was performed by two investigators (J.E. and M.C.) throughout the study. Mucosal lesions on the nasal mucosa and oral cavity were examined with a light, and laryngeal involvement was assessed using a laryngoscope. Each mucosal lesion was evaluated for the presence of erythema, edema, ulceration, and scarring.

**Randomization and treatment.** Eligible patients were randomly allocated to the two study groups using a computer-generated random table in a 1:1 ratio. Patients received aminosidine sulphate (AS: Gabbromicina; Carlo Erba Farmitalia, Milan, Italy), 14 mg/kg body weight, once daily, by intramus-

\* Address correspondence to Carlos Seas, Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima AP 4314, Lima 100. E-mail: cseas@upch.edu.pe

cular injection for 21 days (total dose of 294 mg/kg), or meglumine antimonate (MA: Glucantime; Rhone Poulenc Rorer, Paris, France), 20 mg of pentavalent antimonial/kg body weight in 250 mL 5% dextrose in water infused over a 20-minute period once daily for 28 days. Patients were hospitalized throughout the period of treatment. No other antileishmanial drugs were allowed. A detailed history and complete physical examination was performed on admission. Each day patients were questioned for adverse events and were examined physically. Mucosal lesions were reassessed at the end of treatment and every 3 months for 1 year. Parasitologic examination was repeated if lesions persisted.

**Outcome measurements.** The primary outcome of the study was clinical cure. Patients were considered cured if the lesions appeared completely healed and re-epithelialized, and there were no inflammatory changes visible 1 year after finishing treatment. Clinical improvement was defined as reduction of the observed inflammatory area and no detection of parasites by culture or PCR of a biopsy specimen. Failure was defined as < 50% healing of the mucosal lesion or when clinical improvement was seen but parasites were isolated on culture or PCR was positive. Relapse was defined as enlargement of the initial mucosal lesion or appearance of new lesions in previously spared mucosal or dermal areas after attaining clinical improvement or cure. Failures and relapses were grouped together as failures for the outcome analysis. Secondary outcomes were safety and tolerability. Safety was evaluated with serial laboratory determinations of hematology (including hematocrit, hemoglobin, white blood cell count, and platelets), serum chemistry (creatinine, electrolytes, transaminases, alkaline phosphatase, and total bilirubin), urinalysis, and electrocardiogram all performed at baseline and weekly while on treatment. Tolerability of study drugs was evaluated by daily interviews with patients.

**Statistical analysis.** The sample size was calculated as 48 subjects per study group to detect a difference in clinical cure rates of 30%, estimated as 70% with MA and 40% with AS, with an  $\alpha$  error of 0.05 and a  $\beta$  error of 0.2. The  $\chi^2$  test with continuity correction or Fisher exact test was used to contrast categorical variables. Continuous variables were contrasted with Student *t* test when normally distributed or with the Mann-Whitney Wilcoxon test otherwise. All tests were two-tailed. Data were entered and analyzed using EPI-Info statistical software (version 6.0; CDC, Atlanta, GA).

## RESULTS

**Patient characteristics.** Enrollment was stopped after 38 patients had been enrolled, 21 in the AS group and 17 in the MA group, because of marked clinical differences in the clinical response between the two groups. The comparison of baseline characteristics is shown in Table 1. The two groups were comparable in all respects, including extent of mucosal involvement. All patients were men. More patients with severe laryngeal and vocal cord involvement were enrolled in the AS group, but the difference did not reach statistical significance ( $P = 0.140$ ). Parasitologic confirmation of diagnosis was achieved in all patients (7 by culture and 31 by PCR).

**Outcome evaluation.** Thirty-eight patients were available for determination of clinical outcomes (21 in the AS group and 17 in the MA group). Adherence of patients to post-treatment study visits was good. Cure rates were 47% (8/17;

TABLE 1  
Baseline characteristics

Characteristic	Aminosidine (n = 21)	Meglumine antimonate (n = 17)
Male sex (n)	21	17
Age in years (mean $\pm$ SD)	32.6 $\pm$ 8.4	33.2 $\pm$ 8.3
Weight in kg (mean $\pm$ SD)	55.0 $\pm$ 6.5	55.7 $\pm$ 6.4
Duration of residence in an endemic area in months (mean $\pm$ SD)	20.1 $\pm$ 32.0	19.4 $\pm$ 26.2
Duration of mucosal disease in months (mean $\pm$ SD)	43.3 $\pm$ 52.2	33.2 $\pm$ 26.3
Previous treatment with pentavalent antimonials (n)	10	8
Active cutaneous disease (n)	4	3
Extension of mucosal involvement (n)		
Nose, pharynx, and palate	5	9
Nose, pharynx, palate, and epiglottis	5	4
Nose, pharynx, palate, epiglottis, and vocal cords	11	4
Baseline serum creatinine in mg/dL (mean $\pm$ SD)	0.59 $\pm$ 0.20	0.61 $\pm$ 0.25

95% confidence interval [CI]: 23–71%) in the MA group and 0% (0/21) in the AS group ( $P < 0.001$ ). Cure rates among patients with laryngeal involvement were 37.5% (3/8) in the MA group and 0% (0/16) in the AS group ( $P = 0.065$ ). Clinical outcomes did not correlate with the duration of mucosal disease: 38.7  $\pm$  34.6 months in cured patients versus 38.6  $\pm$  44.3 months in failed patients. Median time to achieve cure in the MA group was 3 months (95% CI: 3–5 months). Mucosal lesions improved during treatment in both groups, but the response was not sustained over time in the AS group. In the nine treatment failures in the MA group, parasitologic confirmation was obtained in five: one by isolation in culture and four by PCR. In the 21 treatment failures in the AS group, parasitologic confirmation was obtained in 15: 6 by isolation in culture and 9 by PCR. Patients not achieving clinical cure were treated with amphotericin B deoxycholate with good results.

**Adverse events and tolerance.** There were no serious adverse events with either drug, nor any differences in the rates of hematologic and serum chemistry abnormalities (Table 2). Transient and mild EKG abnormalities were observed in the MA group that did not need therapeutic intervention. AS was

TABLE 2  
Outcome measurements and safety results

Characteristic	Aminosidine (n = 21)	Meglumine antimonate (n = 17)	P value
Outcome measurements at 1 year of follow-up (n)			
Cure	0	8	
Failure	21	9	< 0.001
Experiencing at least one adverse effect (n)	12	11	0.63
Fever (n)	3	4	0.67
Chills (n)	5	7	0.25
Arthralgia (n)	9	11	0.18
Anorexia (n)	1	4	0.15
Myalgia (n)	8	11	0.10
Serum creatinine at the end of treatment (mg/dL; mean $\pm$ SD)	0.62 $\pm$ 0.24	0.60 $\pm$ 0.16	0.75

associated with pain at the injection site that improved with the application of local heat.

## DISCUSSION

The results of this study indicate that parenteral AS on its own is not effective in the treatment of moderate MCL in Peru. Despite some initial clinical response in the AS group, none of the 21 patients available for outcome determinations achieved clinical cure. In contrast, 47% of patients achieved clinical cure in the MA group, with a 38% cure rate in patients with extensive laryngeal involvement.

Aminosidine has been extensively evaluated for the treatment of CL. Topical preparations containing 15% aminosidine with different vehicles to promote absorption through the skin and to avoid irritation have shown good clinical results.<sup>18–20</sup> Parenteral AS is highly effective for visceral leishmaniasis in Africa and India and for diffuse cutaneous leishmaniasis caused by *L.(L.) aethiopica* in Ethiopia.<sup>17,21,22</sup> However, parenteral AS showed only limited efficacy for new world CL caused by two species. One study conducted in Colombia, in military recruits with confirmed infection by *L.(V.) panamensis* in one third of them, showed cure rates of 50% (total dose of 252 mg/kg) after 1 year of treatment.<sup>25</sup> Similar results were observed in Belize with a slightly higher total dose of intravenous AS (280 mg/kg) in patients infected with *L.(V.) braziliensis*.<sup>26</sup> Clinical efficacy at 6 weeks of treatment was 59% in AS-treated patients compared with 88% in patients treated with a pentavalent antimonial. These results are not surprising given that the *in vitro* susceptibility of species of *Leishmania* to AS vary greatly, with higher susceptibility for *L.(L.) major* and *L.(L.) tropica* (effective dose [ED<sub>50</sub>] in the range of 1–5 µmol/L), intermediate susceptibility for *L.(L.) donovani* (ED<sub>50</sub> = 6–18 µmol/L), and lower susceptibility for *L.(V.) braziliensis* and *L.(L.) mexicana* (ED<sub>50</sub> ranging from < 12 to 39 µmol/L).<sup>27</sup>

The only previous study of aminosidine in MCL was conducted in Brazil and reported good initial response to AS in patients with MCL but marked reduction in cure rates during the follow-up period.<sup>23</sup> AS (total dose of 320 mg/kg, compared with ours of 294 mg/kg) attained 67% (10/21) cure rate after 3 months of finishing the treatment, falling to 48% after 1 year, and 33% after 2 years. No detailed descriptions of the severity of illness or extension of mucosal involvement were provided, so it is not possible to make an accurate comparison of the two studies. Considering these data and the results of our study together, it is clear that parenteral AS alone has limited efficacy for new world CL and MCL caused by *L.(V.) braziliensis*. Possible reasons that might explain these findings are the relative insensitivity of *L.(V.) braziliensis* to AS, pharmacokinetic parameters including inadequate volume of distribution of AS leading to low tissue levels in affected mucosae, and the severity and extension of mucosal involvement. Primary clinical unresponsiveness to AS has only been reported once,<sup>17</sup> with no elucidation of the underlying mechanism.<sup>28</sup> We do not have evidence to postulate that acquired resistance to AS played a role in the lack of efficacy of AS shown in our study. AS induced pain at the injection site in our patients, and better tolerance has been reported in patients with visceral leishmaniasis and diffuse cutaneous leishmaniasis, using even higher doses and for more prolonged periods than in our study.<sup>17,21</sup>

At the present time, parenteral AS should not be used alone to treat MCL in the new world or CL in areas where *Leishmania* remain susceptible to pentavalent antimonials or where *L.(V.) braziliensis* is the dominant organism. A synergistic association of AS with pentavalent antimonials has been suggested *in vitro* and corroborated in a clinical trial performed in patients with visceral leishmaniasis.<sup>29</sup> In that study, two different dose regimens of AS (total doses of 252 and 378 mg/kg) combined with a 21-day course of pentavalent antimonials performed better than a 30-day course of pentavalent antimonials alone. These encouraging results merit study of the combination in MCL. Although the preparation of parenteral AS used in clinical trials is no longer available in the market, a generic compound has been evaluated in visceral leishmaniasis and licensed in India and is expected to become available soon.<sup>30</sup>

We conclude that parenteral AS given on its own is not effective for the treatment of moderate MCL caused by *L.(V.) braziliensis*. The combination of AS and pentavalent antimonials for MCL merits evaluation because new therapeutic options are needed for this highly prevalent, disfiguring, dangerous, and difficult-to-treat condition.

Received January 23, 2007. Accepted for publication February 6, 2007.

Acknowledgments: We thank Julieta Tupayachi for providing technical support in conducting the study.

Financial support: This study was funded by a research grant from the UNDP/WOLRD Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) 930033.

Disclaimer: The authors disclosed no conflict of interest.

Authors' addresses: Alejandro Llanos-Cuentas, Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima AP 4314, Lima 100, Peru, Telephone: 51-1-4827739, Fax: 51-1-4823404, E-mail: allanos@upch.edu.pe. Juan Echevarria, Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima AP 4314, Lima 100, Peru, Telephone: 51-1-4823910, Fax: 51-1-4823404, E-mail: chito@upch.edu.pe. Carlos Seas, Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima AP 4314, Lima 100, Peru, Telephone: 51-1-4823910, Fax: 51-1-4823404, E-mail: cseas@upch.edu.pe. Elsa Chang, Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima AP 4314, Lima 100, Peru, Telephone: 51-1-4823910, Fax: 51-1-4823404, E-mail: echangb@hotmail.com. Maria Cruz, Departamento de Medicina del Hospital Nacional Sur Este-Essalud Cuzco, Perú, Av Juan Anselmo Alvares S/N, Wanchaq, Cuzco-Perú. Telephone/Fax: 51-84-234724, E-mail: mcruzvene@yahoo.com.ar. Eugenia Alvarez, Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima AP 4314, Lima 100, Peru, Telephone: 51-1-4823910, Fax: 51-1-4823404, E-mail: eat@upch.edu.pe. Ernesto Rosales, Hospital Regional del Cuco, Cusco, Peru Jr. Tacna 886, Peru, Telephone: 511-2632549, Fax: 511-2632549, E-mail: ernestorosales@terra.com.pe. Pablo Campos, Facultad de Salud Pública y Administración, Universidad Peruana Cayetano Heredia, Lima, Peru, Telephone: 51-1-3190000, Fax: 51-1-4823404, E-mail: pecampos@upch.edu.pe. Anthony Bryceson, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK, Telephone: 44-1869-331027, Fax: 44-1869-331027, E-mail: a.bryceson@doctors.org.uk.

## REFERENCES

1. Marsden PD, 1986. Mucosal leishmaniasis ("espundia" Escomel, 1911). *Trans R Soc Trop Med Hyg* 80: 859–876.
2. Lucas CM, Franke ED, Cachay MI, Tejada A, Cruz ME, Kreutzer RD, Barker DC, McCann SHE, Watts D, 1998. Geo-



- graphic distribution and clinical description of leishmaniasis cases in Peru. *Am J Trop Med Hyg* 59: 312–317.
3. Guthmann JP, Arlt D, Garcia LM, Rosales M, de Jesus Sanchez J, Alvarez E, Lonlas S, Conte M, Bertolotti G, Fournier C, Huari R, Torreele E, Llanos-Cuentas A, 2005. Control of mucocutaneous leishmaniasis, a neglected disease: Results of a control programme in Satipo Province, Peru. *Trop Med Int Health* 10: 856–862.
  4. Miranda-Verastegui C, Llanos-Cuentas A, Arevalo I, Ward BJ, Matlashewski G, 2005. Randomized, double-blind clinical trial of topical imiquimod 5% with parenteral meglumine antimonate in the treatment of cutaneous leishmaniasis in Peru. *Clin Infect Dis* 40: 1395–1403.
  5. Berman J, 2005. Clinical status of agents being developed for leishmaniasis. *Expert Opin Investig Drugs* 14: 1337–1346.
  6. Berman JD, 1997. Human leishmaniasis: clinical, diagnostic, and chemotherapeutic developments in the last 10 years. *Clin Infect Dis* 24: 684–703.
  7. Croft SL, 2001. Monitoring drug resistance in leishmaniasis. *Trop Med Intern Health* 6: 899–905.
  8. Llanos-Cuentas A, Echevarria J, Cruz M, La Rosa A, Campos P, Campos M, Franke E, Berman J, Modabber F, Marr J, 1997. Efficacy of sodium stibogluconate alone and in combination with allopurinol for treatment of mucosal leishmaniasis. *Clin Infect Dis* 25: 677–684.
  9. Franke ED, Wignall FS, Cruz ME, Rosales E, Tovar AA, Lucas CM, Llanos-Cuentas A, Berman JD, 1990. Efficacy and toxicity of sodium stibogluconate for mucosal leishmaniasis. *Ann Intern Med* 113: 934–940.
  10. Franke ED, Llanos-Cuentas A, Echevarria J, Cruz ME, Campos P, Tovar AA, Lucas CM, Berman JD, 1994. Efficacy of 2-day and 4-day regimens of sodium stibogluconate (pentostam) in the treatment of mucosal leishmaniasis. *Am J Trop Med Hyg* 51: 77–82.
  11. Marsden PD, Llanos-Cuentas EA, Lago EL, Cuba CC, Barreto AC, Costa JM, Jones TC, 1984. Human mucocutaneous leishmaniasis in tres bracos, Bahia-Brazil. An area of *Leishmania braziliensis braziliensis* transmission. III. Mucosal disease presentation and initial evolution. *Rev Soc Bras Med Trop* 17: 179–186.
  12. Harbath S, Burke J, Lloyd J, Evans R, Pestotnik S, Samore M, 2002. Clinical and economical outcomes of conventional amphotericin B-associated nephrotoxicity. *Clin Infect Dis* 35: 120–127.
  13. Andersen EM, Cruz-Salazar ME, Llanos-Cuentas A, Cjuno ML, Echevarria J, Miranda-Verastegui C, Colina O, Berman JD, 2005. Comparison of meglumine antimonate and pentamidine for Peruvian cutaneous leishmaniasis. *Am J Trop Med Hyg* 72: 133–137.
  14. Velez I, Agudelo S, Hendricks E, Puerta J, Grogl M, Modabber F, Berman J, 1997. Inefficacy of allopurinol for Colombian cutaneous leishmaniasis: a randomized, controlled trial. *Ann Intern Med* 126: 232–236.
  15. Navin TR, Arana BA, Arana FE, Berman JD, Chajon JF, 1992. Placebo-controlled clinical trial of sodium stibogluconate (pentostam) versus ketoconazole for treating cutaneous leishmaniasis in Guatemala. *J Infect Dis* 165: 528–534.
  16. Mattock NM, Peters W, 1975. The experimental chemotherapy of leishmaniasis II. The activity in tissue culture of some antiparasitic and antimicrobial compounds in clinical use. *Annals of Tropical Medicine and Parasitology* 69: 359–371.
  17. Teklemariam S, Hiwot AG, Frommel D, Miko TL, Ganlov G, Bryceson A, 1994. Aminosidine and its combination with sodium stibogluconate in the treatment of diffuse cutaneous leishmaniasis caused by *Leishmania aethiopica*. *Trans R Soc Trop Med Hyg* 88: 334–339.
  18. El-On J, Halevy S, Grunwald MH, Weinrauch L, 1992. Topical treatment of old world cutaneous leishmaniasis caused by *Leishmania major*: a double-blind control study. *J Am Acad Dermatol* 27: 227–231.
  19. Soto J, Toledo JT, Gutierrez P, Arboleda M, Nicholls RS, Padilla JR, Berman JD, English CK, Grogl M, 2002. Treatment of cutaneous leishmaniasis with a topical anti leishmanial drug (WR279396): phase 2 pilot study. *Am J Trop Med Hyg* 66: 147–151.
  20. Soto J, Hernandez N, Mejia H, Grogl M, Berman J, 1995. Successful treatment of new world cutaneous leishmaniasis with a combination of topical paromomycin/methylbenzethonium chloride and injectable meglumine antimonate. *Clin Infect Dis* 20: 47–51.
  21. Jha TK, Oliario P, Thakur CPN, Kanyok TP, Singhania BL, Singh IJ, Singh NPK, Akhoury S, Jha S, 1998. Randomised controlled trial of aminosidine (paromomycin) v sodium stibogluconate for treating visceral leishmaniasis in North Bihar, India. *BMJ* 316: 1200–1205.
  22. Thakur CP, Kanvok TP, Pandey AK, Sinha GP, Messick C, Oliario P, 2000. Treatment of visceral leishmaniasis with injectable paromomycin (aminosidine). An open-label randomized phase-II clinical study. *Trans R Soc Trop Med Hyg* 94: 432–433.
  23. Romero GAS, Lessa HA, Orge MGO, Macedo VO, Marsden PD, 1998. Mucosal leishmaniasis treatment with aminosidine sulphate: results of two-year follow-up. *Rev Soc Bras Med Trop* 31: 511–516.
  24. Lopez M, Inga R, Cangalaya M, Echevarria J, Llanos-Cuentas A, Arevalo J, 1993. Diagnosis of Leishmania using the polymerase chain reaction: a simplified procedure for field work. *Am J Trop Med Hyg* 49: 348–356.
  25. Soto J, Grogl M, Berman J, Oliario P, 1994. Limited efficacy of injectable aminosidine as single-agent therapy for Colombian cutaneous leishmaniasis. *Trans R Soc Trop Med Hyg* 88: 695–698.
  26. Hepburn NC, Tidman MJ, Hunter JAA, 1994. Aminosidine (paromomycin) versus sodium stibogluconate for the treatment of American cutaneous leishmaniasis. *Trans R Soc Trop Med Hyg* 88: 700–703.
  27. Neal RA, Allen S, McCoy N, Oliario P, Croft SL, 1995. The sensitivity of Leishmania species to aminosidine. *J Antimicrob Chemother* 35: 577–584.
  28. Croft SL, Sundar S, Fairlamb AH, 2006. Drug resistance in leishmaniasis. *Clin Microbiol Rev* 19: 111–126.
  29. Thakur CP, Kanvok TP, Pandey AK, Sinha GP, Zaniewski AE, Houlihan HH, Oliario P, 2000. A prospective randomized, comparative, open-label trial of the safety and efficacy of paromomycin (aminosidine) plus sodium stibogluconate versus sodium stibogluconate alone for the treatment of visceral leishmaniasis. *Trans R Soc Trop Med Hyg* 94: 429–431.
  30. Oliario PL, Guerin PJ, Gerstl S, Haaskjold AA, Rottingen JA, Sundar S, 2005. Treatment options for visceral leishmaniasis: a systematic review of clinical studies done in India, 1980–2004. *Lancet Infect Dis* 5: 763–774.